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Isolation and Screening of Oraganophosphorous Pesticides (Malathion) Degrading Organisms from Soil

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Abstract: Organophosphate like Malathion is used for control of household and agricultural pests. High levels of malathion contaminates soil, water and aquatic ecosystems. The wide spread use of these pesticides over the years has resulted in problems caused by their interaction with the biological systems in the environment. Present study reports the isolation, morphological and Estimation of Malathion degrading bacteria. Soil samples collected from various agriculture field, Garage field, Garden, etc (Vapi and Valsad Region). Malathion was used as a sole source of carbon to enumerate Malathion degrader. Total 15 isolates (AK1…AK15) were obtained from the soil sample, using Mineral Salt Medium.AK5 isolate as shows maximum percentage degradation at 100 ppm concentration of Malathion. The analysis shows that isolated organism belongs to Bacillus species.

Keywords: Malathion, Organophosphate(OP), Biodegradation

I. INTRODUCTION

Pesticides are organic compounds manufactured and used control destructive pests such as insects, plant disease organism , unwanted species of plants and animals causing harm during or otherwise interfering with the production, processing, storage , transparent of marketing of food, agriculture commodities, wood and wood products or animal food stuff, or substances which may be administered to animals for the control of insects, arachnids or other pests in or on their bodies (S.R.Shinde et al., 2015).

Pesticides are indispensible to modern agriculture. Some of the main agricultural products are parathion, methyl parathion, chlorpyriphos, malathion, monocrotophos, quinalphos and dimethoate. Microorganisms are used in bioremediation of environmental pollutants. Bacteria and fungi are capable of degrading malathion. Malathion can be degraded or detoxified using physical, chemical, or biological methods (A.RatnaKumari et al., 2012). Few bacterial species like Bacillus spp., Pseudomonas spp., Staphylococcus spp., Enterobacter spp., Klebsellaspp noted to degrade malathion in recent studies.(Karishma Baishya et al., 2015)

Biodegradation is common method for the removal (degradation and detoxification) of organophosphate pesticides because of its low cost and low collateral destruction of indigenous animal plant organisms (Liu et al., 2007). Bacterial degradation is considered to be a major factor determining the fate of Malathion and other organophosphate pesticides in the environment.Degradation of pesticides is usually a combination of a number of processes, including microbial degradation and chemical hydrolysis and is also influenced by some physicochemical properties such as temperature, pH and carbon and nitrogen source(Soni Yadav et al., 2015).

II. MATERIALS AND METHOD

A. Screening, Isolation and Purification of Malathion Degrading Bacteria

For isolation of malathion degrading bacteria, one gram of each soil sample were suspended into 9 ml of sterile distilled water and serially diluted up to 6 folds. From the last three dilution tubes take 0.1 ml and spread into the mineral salt agar medium containing malathion as sole source of Carbon and Nitrogen. Plates were incubated at 37^0C for 48 hours. The isolated colonies were grown on BHM agar plate.

B. Storage and Maintenance of Pure Culture

The isolated organisms were streaked on MSM agar slants. The slants were incubated at 30°C for 48 hrs. The pure culture is then stored in refrigerator at 4°C and sub cultured periodically

C. Degradation Experiment

1) *Inoculum Preparation:* Inoculum was prepared by transferring preserved culture and growing the cells in 100 ml Erlenmeyer flask containing 50 ml nutrient broth. The flasks were incubated at 30°C for 24 hrs. The freshly grown 24 hrs old culture with 1.0 O.D. at 600 nm is used as Inoculum to inoculate degradation medium MSM broth containing 100 ppm malathion.

D. Media for Degradation

Composition of medium for degradation [K_2HPO_4 6.0gms, KH_2PO_4 1.0grm, NH_2NO_3 1.0gm, $MgSO_4.{}_7H_2O$ 0.1gm, NaCl 5.0 in 1 liter of distilled water ,pH of medium was adjust to 7.0 using pH meter, and medium was autoclaved] containing 100ppm malathion

E. Inoculation of Medium for Degradation

The sterilized medium was inoculated with 100 ppm malathion and 1% (v/v) of 24 hrs old culture. The inoculated flask was allowed to incubate at 30°C for 48 hrs. The sample was withdrawn at 24 hrs of interval and supernatant was subjected to centrifugation at 5,000 rpm for 20 min and degradation rate was determined

F. Estimation of Malathion

1ml of sample,1 ml of alcoholic potassium hydroxide and 10 ml of 0.1N potassium bromate was added. Then 0.5ml of 1:1 nitric acid and 2ml of double distilled water are added to give orange yellow color. The solution was kept aside for 5 min before taking absorbance and absorbance was measured at 415nm against reagent blank.*(Eurasian J Anal Chem 8(3):131-135,*2013*).* Degradation was quantitatively analyzed by measuring the absorbance of the supernatant at maximum absorption wavelength, λmax of respective pesticides. Degradation was calculated by using the equation:

$$\% \text{ Degradation} = (A - B)/A \times 100$$

Where, A is initial absorbance of control malathion (initial absorbance) and B is observed absorbance of degraded malathion (final absorbance).

G. Screening of Isolates having Degradation Activity

Bacteria were screened on the basis of degradation capability using malathion. Bacteria which shows higher degradation activity is selected and further study is carried out using it. The screening of isolates degrading malathion was measured as decrease in optical density using spectrophotometer. Degradation of malathion was done by promising isolate and effect of incubation period was optimized

H. Effect of Incubation Temprature

In present study, the effect of incubation period was determined by incubating medium at different incubation temprature such as 15-45° C. In Erlenmeyer flask, 100 ml of MSM broth, 100 ppm malathion and 1% inoculum were added and incubated for 48 hr. The sample was withdrawn from medium at respective time interval and subjected to centrifugation at 5,000 rpm for 20 min and the supernatant was used for determination of degradation.

III. RESULT AND DISCUSSION

Total 25 isolates were screened for its capability of degrading Malathion. 15 isolates capable of degrading Malathion were isolated from soil samples with a history of pesticide application. All these isolates were labeled as AK-1 to AK-15.All 15 isolates were further purified and stored after streaking on MSM agar plate. Bacterial isolate AK5 showed maximum degradation (76.54%) of Malathion within 48 hrs of incubation (Fig. 3.1) at 30°C under static condition. Followed by AK5, bacterial isolate AK2 & AK8 shows 70.0 % &69.98% phenol degradation, respectively. Thus, isolate AK5 was selected for further study as it exhibit highest degradation of Malathion.

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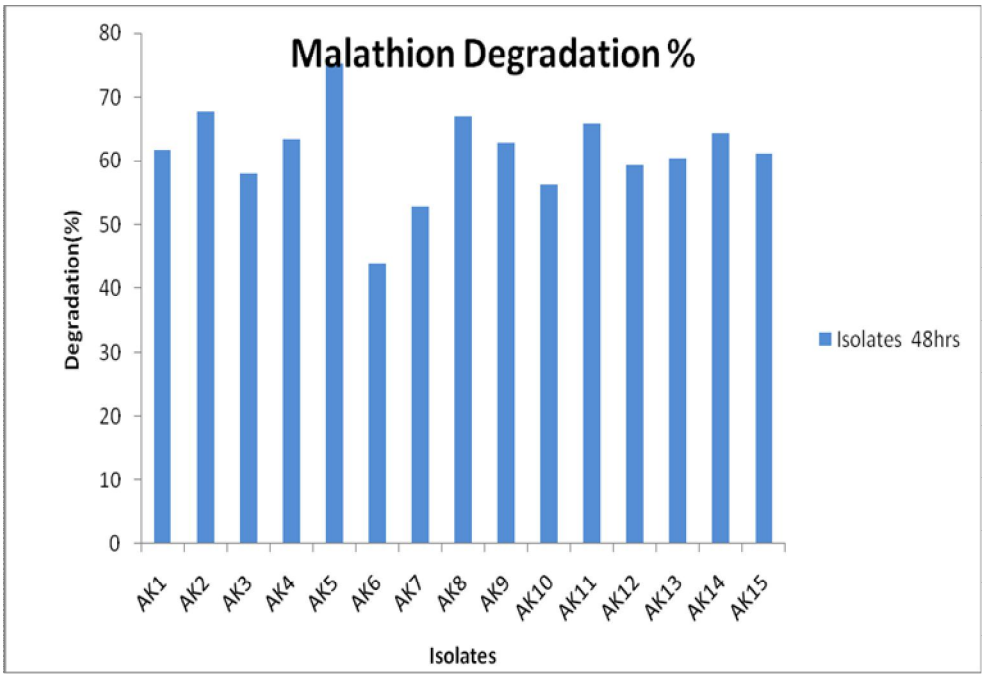

Figure-1 Malathion Degradation

Temperature is one of the important factors affecting the growth and activity of microorganisms. Effect of varying temperature on Malathion degradation was studied by incubating the inoculated experimental flask in the temperature range of 15- 45° C. The sample was with drawn after 48 hrs of incubation, centrifuged at 5000 rpm for 20 min and supernatant was used to determine Malathion degradation. The optimum temperature for maximum degradation of Malathion was recorded at 30° similar result have been repoted by C.Sony Yadav et al., (2015) and KayLynn Newhart et al.,(2006).

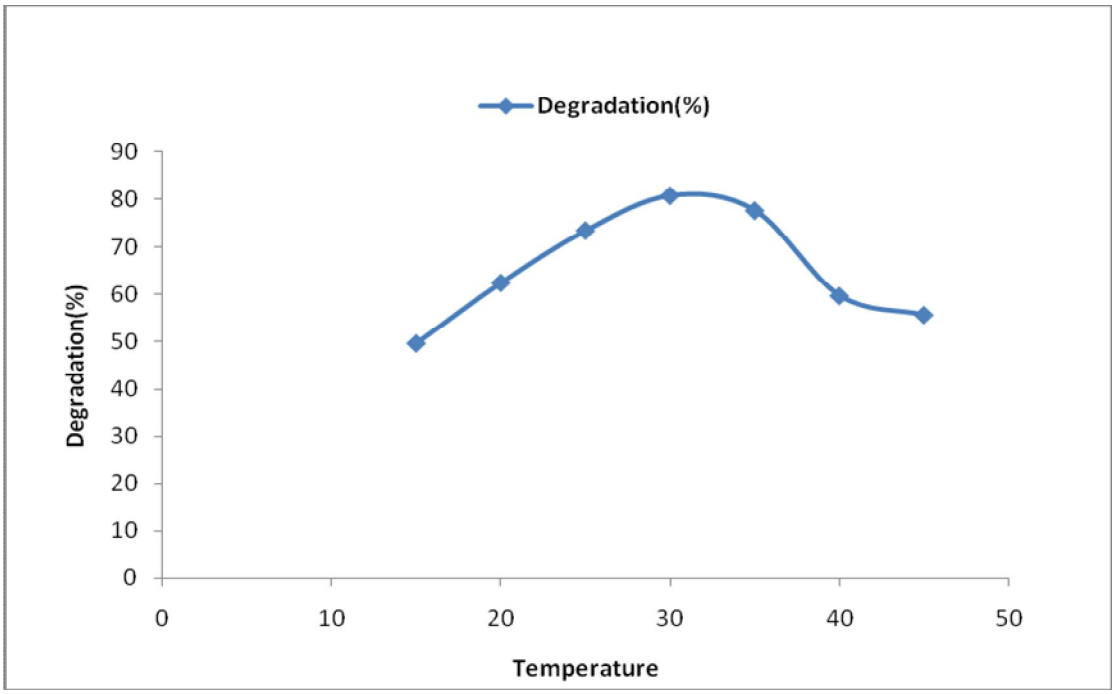

Fig Effect of Temperature on Malathion degradation

IV. CONCLUSION

The study shows that the isolated bacterium AK5 is able to degrade the Malathion, so that this organism is used as a biological agent for the *insitu* bioremediation of Malathion contaminated soil. The present study is done only on the morphological and cultural characterisation of isolated bacteria. These eco-friendly bacteria can be used and motivate the farmers to use natural biological pesticides.

V. ACKNOWLEDGEMENT

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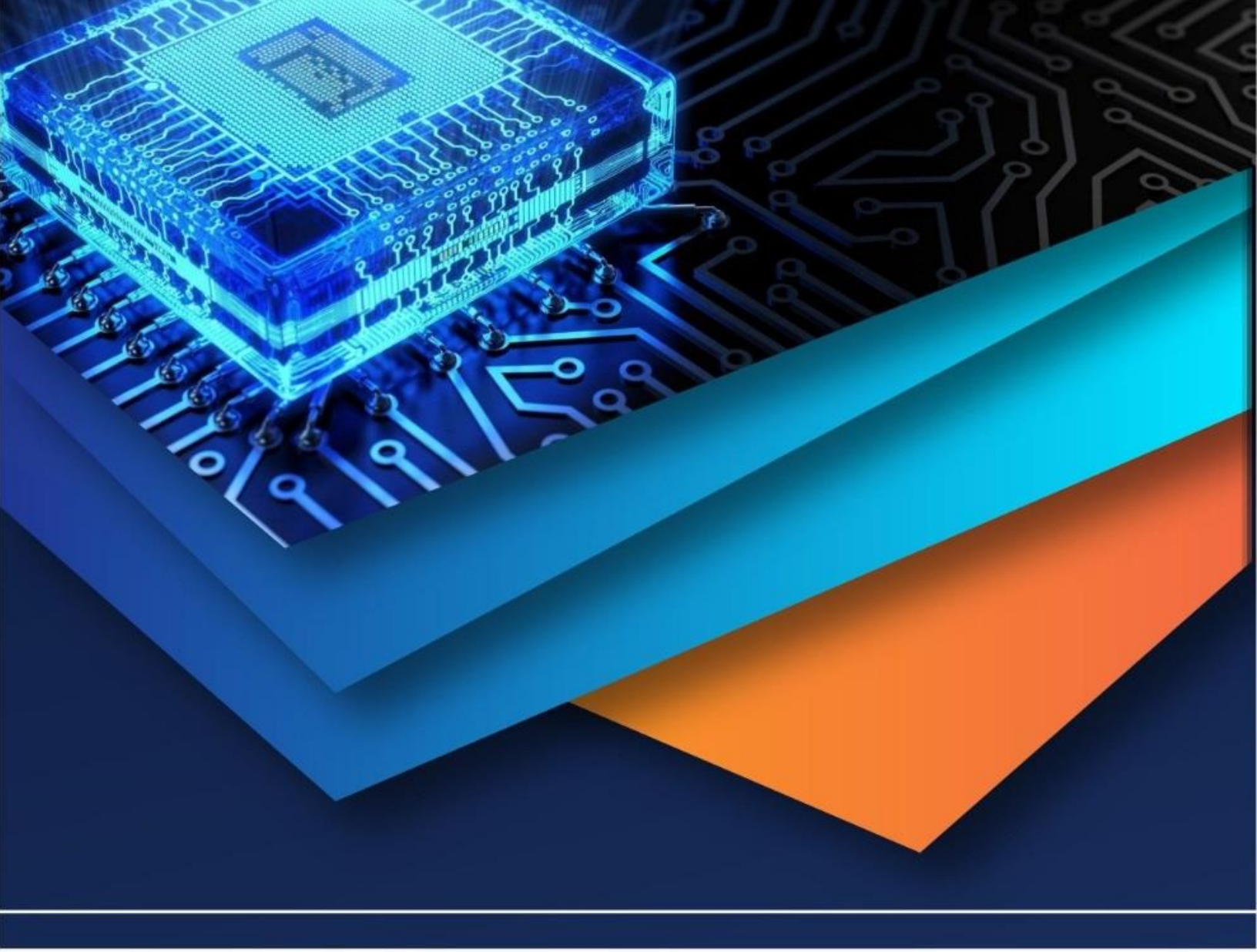

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